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Toxicokinetic Principles in Relation to Percutaneous Absorption and Cutaneous Toxicity

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At the foundation of the sciences of toxicology and pharmacology is the concept that coherent dose-response relationships exist for describing effects of chemicals on biological organisms. In one of several forms, dose-response curves for quantal effects are constructed by plotting the proportion of animals in a group responding with a particular effect on a y-axis, e.g., a dermal effect such as chloracne, versus the logarithm of dose on the x-axis. The resulting curve (Fig. 1) has a sigmoidal shape and usually progresses smoothly from no response at low concentration to 100% response at sufficiently high concentration. Although this is the usual shape for quantal dose-effect relationships, curves with anomalous shapes are not unknown, and such behavior usually gives significant information about mechanisms of the biological effect under investigation (1).

These dose-response curves are deceptively straightforward, but their validity depends on a number of important underlying assumptions. These assumptions are: (a) there is a causal relationship between the applied chemical and the observed

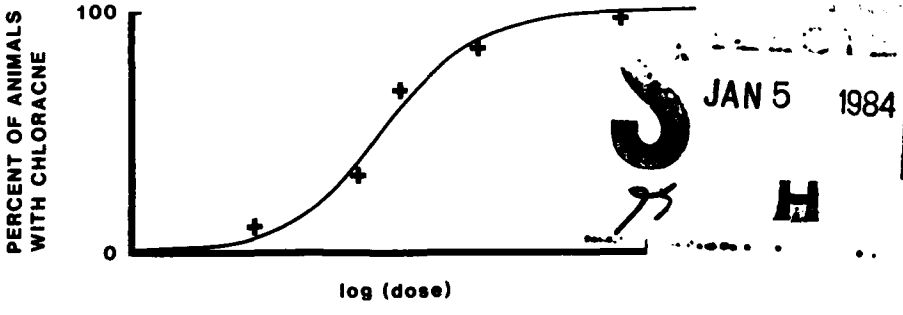

FIG. 1. Typical dose-response curve for a quantal toxic response. A number of test animals were treated with a given dose of test chemical and the percentage of animals exhibiting a particular toxic response calculated. The effect is quantal when the determination is simply whether or not an animal develops the effect. In this example the theoretical effect is developing chloracne; for LD_{50} determinations the quantal response is death.

effect; (b) there is a target tissue which the chemical must reach to elicit the response; (c) the intensity of the response is proportional to the concentration (or for toxicity more usually the concentration-time integral) of the chemical at the target tissue; and (d) the concentration or concentration-time integral of the chemical at the target tissue is directly related to the administered dose. One important goal of toxicokinetic research is direct evaluation of this last assumption. Is it indeed always correct that concentration or concentration-time integrals at target tissues are directly related to dose, especially over very large ranges of dose? In toxicokinetic research the relationship between the dose to the target tissue and the administered dose, or between the toxicity itself and the dose at the target tissue, can be examined quantitatively.

Toxicokinetics, as used here, refers to the detailed description of the time course of the chemical within various tissues in test animals (Fig. 2). This includes analysis of the processes of absorption, distribution, metabolism, and elimination of chemicals and their metabolites. In contrast, toxicodynamics is the relationship between toxicokinetic behavior and some toxicological response. Optimally, toxicokinetic and toxicodynamic research should proceed hand in hand, so observed toxic effects can be quantitatively related to some measure of the dose to the target organ. In toxicology the important measure of dose is usually the concentration-time integral, which is the area under the curve (AUC) of local target concentration versus time. Evaluation of the delivered dose at various tissues has been referred to as dosimetry. These definitions follow the suggestions of Young and Holson (23) from their review on the utility of pharmacokinetics for research in chemical teratology.

Narrowing the focus to discuss dermal toxicity, we can identify a variety of toxicodynamic parameters related to skin tissue. Irritation is the result of direct caustic action of chemical applied to the skin. Dermal toxicity, as used more broadly in this chapter, is defined as an action of a chemical (or a metabolite) on the skin,

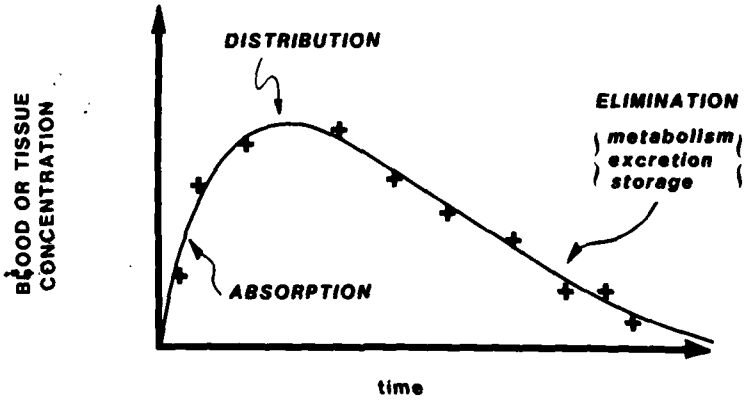

FIG. 2. Typical time course for a chemical in the blood or a target tissue. The curve demonstrates processes of increasing concentration during absorption and then elimination as the chemical is lost by a variety of physiological processes.

either irritative or nonirritative in nature, or a deleterious action on skin tissue from a chemical delivered by a route other than dermal application. Phototoxicity is a toxic effect which requires the presence of a chemical or metabolite in skin tissue plus radiant energy. Two other effects—sensitization and photoallergy—are mediated via the immune system and are not discussed in any detail in this chapter. The main goal here is to evaluate some basic toxicokinetic principles as they relate to the skin in its dual role as a target organ and an organ of delivery.

COMPARTMENTAL DESCRIPTIONS AND SOME KINETIC TERMINOLOGY

Hydrazine has been used as a torpedo propellant and a missile fuel. More recently it has found application as the fuel source for emergency propulsion units in jet aircraft. Because there may be skin exposure of workmen during maintenance of these power units, studies were conducted to determine the percutaneous absorption and toxicokinetics of hydrazine following dermal application.

Hydrazine toxicity has both a local aspect, with skin as the target organ for its irritant action, and a systemic aspect, with skin acting as a barrier to absorption. To produce systemic toxicity, hydrazine must penetrate the skin, enter the blood, and reach the liver and brain, the two major target organs.

The principles of compartmental kinetics as applied to bioavailability are widely used. The typical bioavailability study deals with gastrointestinal or parenteral absorption of a material rather than percutaneous absorption. A study done by us (12) in which H-70, an azeotropic hydrazine solution (70% hydrazine/30% H_2O), was administered percutaneously to rabbits provides an example for outlining some principles of compartmental toxicokinetics as they apply to percutaneous toxicity and bioavailability.

Percutaneous exposures were accomplished by fixing a piece of fiber glass screen to a shaved area on the side of each rabbit. This screen ensured even distribution of fluid over the skin and a close correlation between dose-volume and skin area exposed. To prevent the fluid from being dislodged, a styrofoam donut with stainless steel screen cover was fixed in place over the treated area. Unlike most studies of dermal absorption of volatile chemicals (11), no attempt was made to impede evaporation by covering the treated area. To obtain an estimate of evaporative loss, volumes of hydrazine similar to those placed on the rabbit skin were placed on fiber-glass-screen-covered glass slides, which were periodically weighed to determine the rate of fluid loss by evaporation (Fig. 3).

The time course of hydrazine in rabbit blood following percutaneous application (Fig. 4) demonstrates a period of absorption, attainment of a maximum concentration, and elimination. A relatively simple kinetic model was used to describe hydrazine absorption and elimination (Fig. 5). Hydrazine applied to the skin surface is lost by evaporation and by absorption through the skin into the blood. Once absorbed into the blood it is then lost from the body by elimination with a rate constant, k_{el}. All processes are regarded as first order; that is, the rate of change

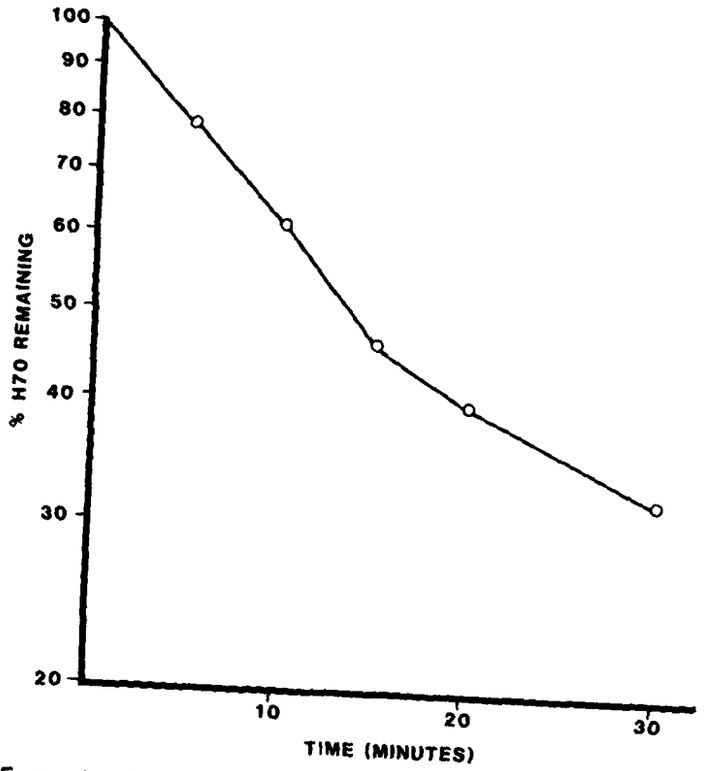

FIG. 3. Evaporation of azeotropic hydrazine (H-70) from a glass surface at 22°C. The *in vitro* evaporation constant (k_w) is 3.2 hr^{-1} for the early phase of evaporation. Data are plotted semilogarithmically.

is given by a first-order rate constant times a concentration term. For instance, loss of hydrazine from the blood is defined mathematically as:

$$\frac{-d}{dt}(\mathrm{Hz})_{\mathrm{blood}} = k_{el}(\mathrm{Hz})_{\mathrm{blood}} \qquad [1]$$

The constant k_{el} can be determined in the absence of absorptive processes. This is done by administering hydrazine intravenously (Fig. 4). Elimination rates are then evaluated without complications from any absorptive phase.

It is a property of Eq. 1 that an integrated form

$$\ln \frac{(\mathrm{Hz})_t}{(\mathrm{Hz})_0} = -k_{el}t \qquad [2]$$

gives a straight line with a slope equal to $-k_{el}$ when plotted semilogarithmically (Fig. 6).

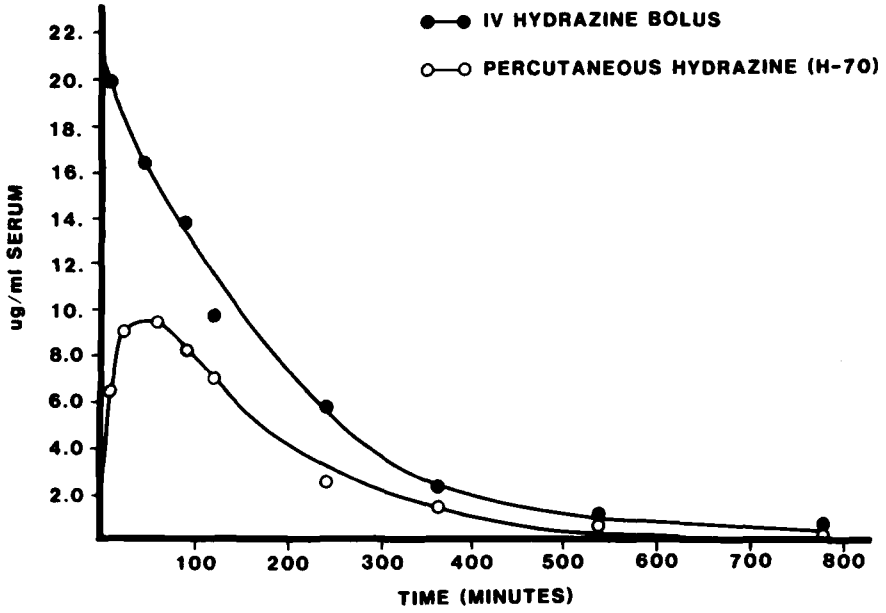

FIG. 4. Percutaneous hydrazine absorption and elimination. All animals received a hydrazine dose of 12 mg/kg. Data points are mean values of four rabbits.

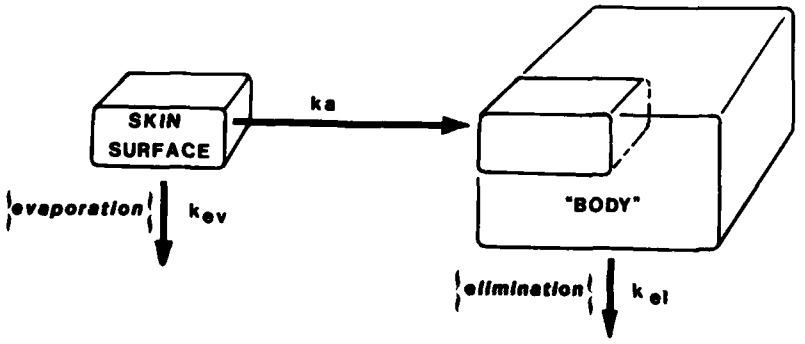

FIG. 5. Compartmental model for hydrazine absorption. All processes—evaporation, uptake, and elimination—are assumed to be first order. The box-within-a-box depiction is used to indicate that hydrazine is absorbed into the blood (small box), but hydrazine in blood is in rapid equilibrium with other portions of the body. This figure then indicates that the volume of distribution of hydrazine is larger than the blood volume.

$$\ln (\mathrm{Hz})_t = \ln (\mathrm{Hz})_0 - k_{el}t \qquad [3]$$

For hydrazine, k_{el} was 0.29 hr^{-1}. Another property of a first-order process is that the time required to reduce the concentration by half is always constant. This is termed the half-life and is

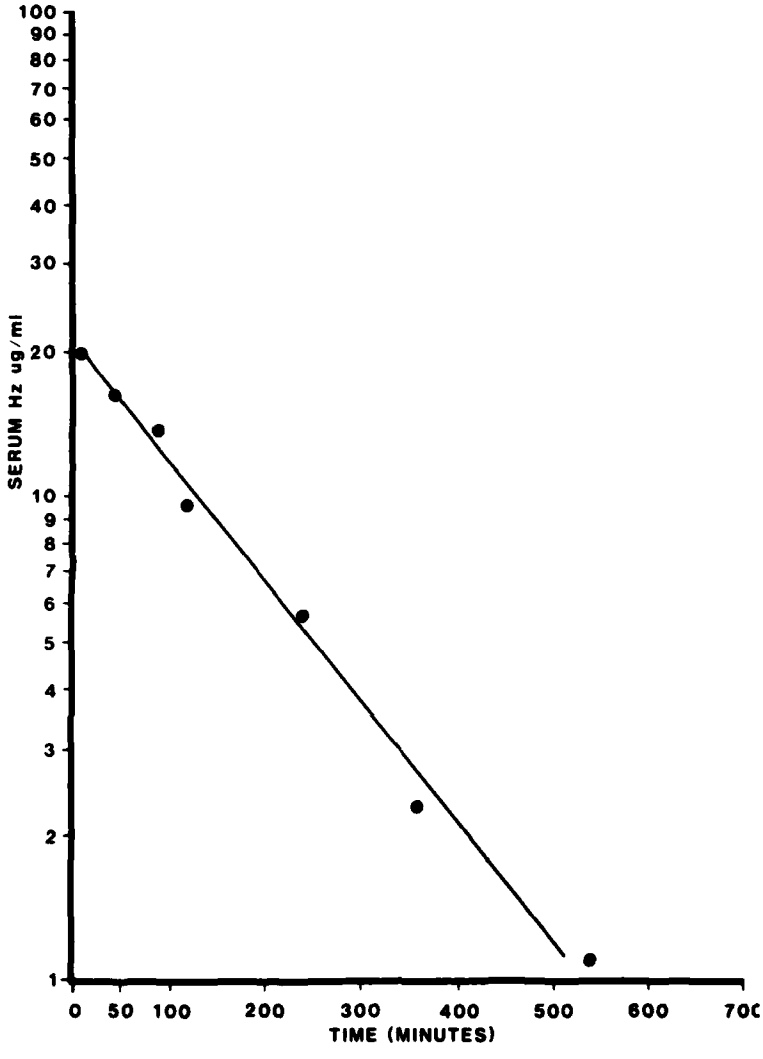

FIG. 6. First-order elimination of hydrazine following intravenous administration. This is a semilogarithmic plot of serum hydrazine data as a function of time after dosing. Points are mean values from five rabbits. k_{el}, 0.29 hr^{-1}; $t_{1/2}$, 2.3 hr.

$$t_{1/2} = \frac{\ln 2}{k_{el}} \qquad [4]$$

For hydrazine, the half-life for disappearance from the blood after intravenous injection is 2.3 hr.

The behavior of hydrazine is such that the body acts as a single homogeneous compartment for its elimination. The behavior of the other components of the body

compartment is inferred by measuring hydrazine in blood. The distribution of hydrazine is not necessarily limited to blood alone. In this regard, intravenous blood data provide information on how extensively the chemical is distributed. For a given amount of hydrazine placed in the body compartment, there is an increase in plasma concentration. The increase is proportional to dose but should be inversely proportional to the volume throughout which the dose distributes.

$$\text{Concentration} = \text{dose/VD} \quad [5]$$

$$\text{VD} = \text{dose/concentration} \quad [6]$$

This parameter, called the apparent volume of distribution (VD), is a virtual volume into which the dose would have to have been placed to yield the observed concentration increase. For hydrazine, the VD is 630 ml/kg.

Bioavailability is a term used to describe the extent to which a given dose of test compound is absorbed. If we assume that an intravenous dose has maximum bioavailability, it is a simple task to determine the extent of absorption following some other route of administration. This is done by giving equivalent doses by both intravenous and dermal routes and calculating the total dose to the body compartment as the concentration-time integral. Then bioavailability becomes:

$$\text{Bioavailability} = \% \text{ absorbed} = \frac{\text{AUC dermal}}{\text{AUC } \textit{intravenous}} \quad [7]$$

For hydrazine the bioavailability is 55% when given as the H-70 water-azeotrope.

Once the kinetic behavior of the intravenous dose has been determined, the constant rate of the absorption phase can be estimated by feathering (Fig. 7). With hydrazine, the k_a value estimated by this technique is 3.47 hr^{-1}. Other methods, e.g., that described by Wagner and Nelson (21), are also available for estimating these kinetic constants. Lastly, we can use basic relationships from chemical kinetics to estimate k_{ev} for our simple model. For competing first-order processes, the fraction absorbed is given by:

$$\text{Fraction absorbed} = \frac{k_a}{k_a + k_{ev}} = \frac{\text{AUC dermal}}{\text{AUC intravenous}} \quad [8]$$

Substituting k_a and the fraction absorbed yields an estimated k_{ev} of 2.9 hr^{-1}. This estimated evaporation constant (k_{ev}) is a composite of simple evaporative loss, evaporation from the epidermal compartment, and hydrazine lost to epidermal tissue binding, metabolism, and other interactions. Because the experimentally measured rate constant for the early phase of *in vitro* evaporation is similar (3.2 hr^{-1}), this simple model with evaporation competing with absorption seems adequate to describe percutaneous absorption of the H-70 hydrazine azeotrope in the rabbit.

The adequacy of this model was tested by treating groups of animals with hydrazine and terminating the dermal exposure by neutralizing the hydrazine at ap-

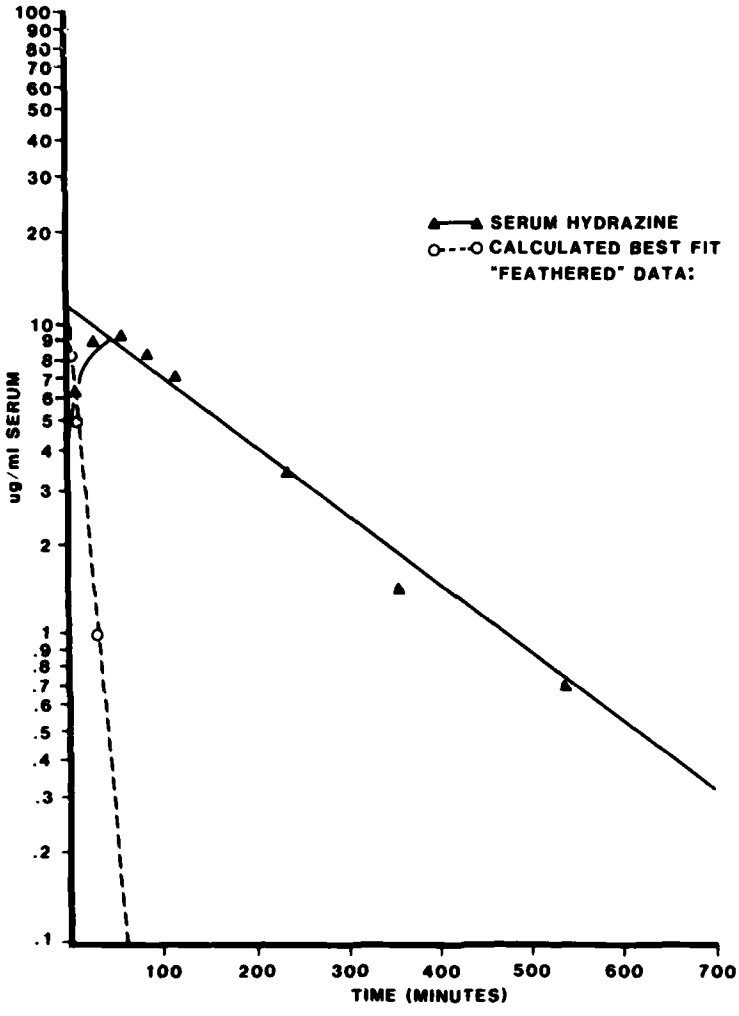

FIG. 7. Estimating the rate constant for absorption of hydrazine by feathering. Triangles are the semilogarithmic plot of serum hydrazine data following dermal application. Feathering is accomplished by fitting a line through the terminal portion of the semilogarithmic plot which gives maximum weight to the elimination phase of the curves. This line is extrapolated to time zero, and each experimental absorption phase data point is subtracted from it. The value for k_a is estimated from the dashed line, which is a semilogarithmic plot of the difference between the experimental values and the extrapolated values (from the solid extrapolated line). Feathering allows the curve to be graphically separated into its two component parts: an elimination phase *(solid line)* and an absorption phase *(dashed line)*.

propriate time intervals (13). The percent dose absorbed in these limited-time exposure groups can be determined by comparison of AUCs with the AUC for a group where the H-70 is left on without washing. The percent dose absorbed verus time before washing can be graphed (Fig. 8). This experimentally derived curve can be compared

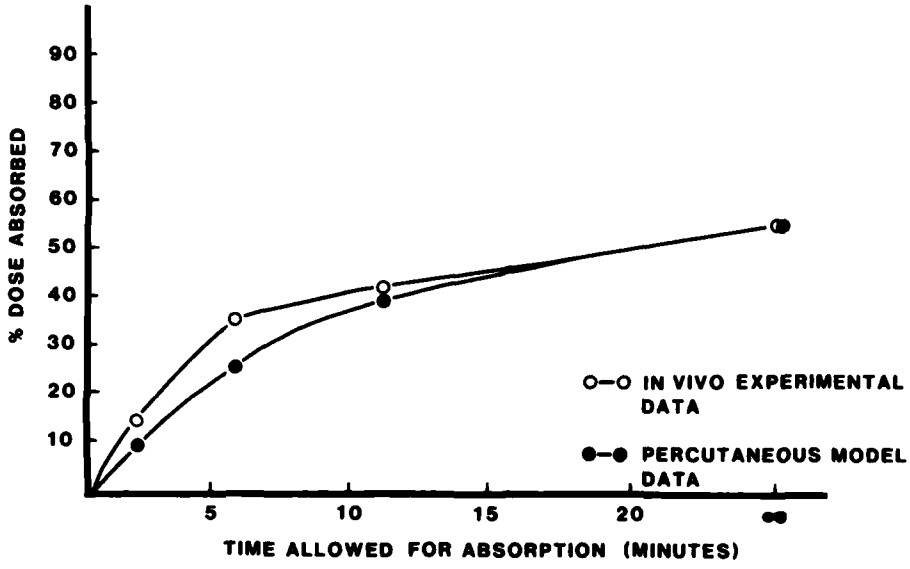

FIG. 8. Open circles represent the percent dose absorbed from percutaneous hydrazine applications of 12 mg/kg followed by removal of hydrazine at 2, 6, and 12 min. Points are mean values of four rabbits. Solid circles are derived by substituting time at the appropriate intervals into Eq. 9, which used the k_a and k_{ev} determined experimentally to predict percent dose absorbed at each time interval.

with a model curve generated by substituting time at the appropriate intervals into Eg. (9), which was described in Notari (16).

$$\ln (A_\infty - A_t) = \ln A_\infty - (k_a - k_{ev})t \qquad [9]$$

Here A is the experimentally determined percent dose absorbed when no washing is attempted, and k_a and k_{ev} are the experimentally derived absorption and evaporation constants. This model predicts an amount of percutaneous absorption only slightly lower than that actually observed.

PHYSIOLOGICAL MODELS

This simple model for hydrazine kinetics defines the time course of hydrazine in blood with several rate constants and the apparent VOD. These parameters in themselves are now predictive of hydrazine absorption and elimination in the rabbit, but they lack direct physiological reality.

Throughout the remainder of this chapter another modeling approach is outlined which is more physiological in character. True physiological models describe the body in terms of anatomically realistic compartments (organs) which are described with relation to blood flow, volume, and solubility characteristics for the test agent. These models have been applied to the study of pharmacokinetics of inhaled gases and vapors (15,18) and a variety of cancer chemotherapeutic agents (10). They

have not been applied directly to the problem of skin absorption of xenobiotic agents, but such a program is now in progress in our laboratory.

When one considers even a relatively simple model for skin absorption (Fig. 9), some of the variables which influence kinetic behavior become apparent. The rate of uptake, defined above by a single constant, is complexly related to the area of skin involved, the blood flow from that region, and skin characteristics in terms of permeability and thickness. The volume of distribution becomes a weighted parameter where storage in each tissue (equilibrated with blood concentrations) contributes to establishing a given blood concentration. Physiological descriptions are also useful in providing specific evaluation of individual body regions and examining behavior of these regions separately, whenever sufficient data exist on tissue levels, etc. Futhermore, a homogeneous body model may be adequate if all tissues are equilibrated with the xenobiotic agent in blood, but this is not always true. It is possible that diffusion limits the movement of the chemical into the tissue, and no consistent relationship would necessarily be expected between tissue and blood levels (14).

DERMAL APPLICATION: DERMAL TOXICITY (IRRITATION)

There are various combinations of application and toxicity to consider where the skin may or may not be the route of entry and may or may not be the target. In the first combination, irritation, the skin is both route of entry and target. With irritation (Fig. 10), there are local, direct effects of a topically applied chemical.

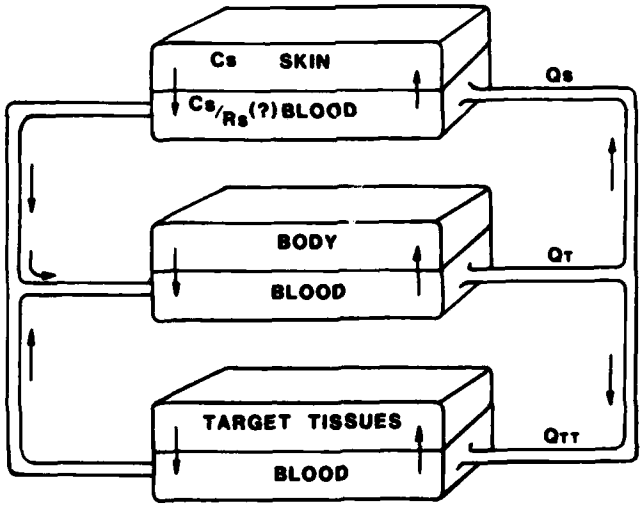

FIG. 9. Simplified physiological model for skin absorption. Realistic values for blood flow, organ volumes, and chemical partitioning are used in evaluating physiological models. The body other than skin (route of entry) and target tissues is lumped into a single compartment here. Q_s, Q_T, and Q_{TT} are, respectively, blood flow to skin, total cardiac output, and blood flow to target tissue. C_s is the concentration of chemical in skin tissue, and R_s is the skin/blood partition coefficient for the chemical.

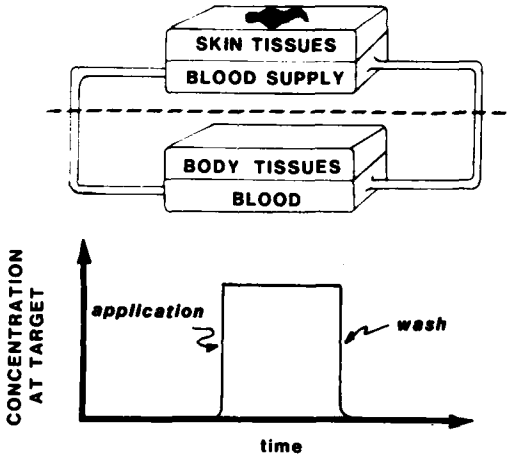

FIG. 10. Irritation. Skin is both the site of application and the target tissue.

In its simplest form, exposure can be depicted as applying a given concentration of the agent to the skin, leaving it there for some time, then washing off the residual. The skin concentration versus time curve is a step function. It is relatively easy to see that the concentration-time cross product at the skin surface is proportional to the concentration. This integral should also be proportional to the applied dose when the chemical remains in contact with the skin until it is all absorbed or removed by combined first-order processes as might occur with volatile xenobiotics, e.g., hydrazine.

In this case, the amount (Amt) on the skin surface is expected to decline exponentially with a combined rate constant equal to the sum of the individual rate constants ($\Sigma_i k_i$), i.e., evaporation, absorption, etc. Then

$$(\text{Amt})_t = (\text{Amt})_0 \exp(-\Sigma_i k_i t) \quad [10]$$

and

$$\text{AUC} = \int_0^\infty (\text{Amt})_t = (\text{Amt})_0/\Sigma_i k_i \quad [11]$$

This formulation is not rigorously correct in that it describes the amount on the surface and not the concentration.

SYSTEMIC TOXICITY: DERMAL APPLICATION

With systemic toxicity caused by dermal application, similar considerations apply when estimating delivered dose at the skin surface, but here delivery of a toxic chemical to a target tissue (Fig. 11) remote from the site of application is the more important concept. Although it probably is not a major problem with dermal application, there is a potential loss of chemical in the skin tissues by metabolism, which is a form of "presystemic clearance." It is reported that over 80% of dermally applied nerve toxin Soman is metabolized during passage through human skin (20). There is also the potential for "presystemic clearance" in pulmonary tissues, as all

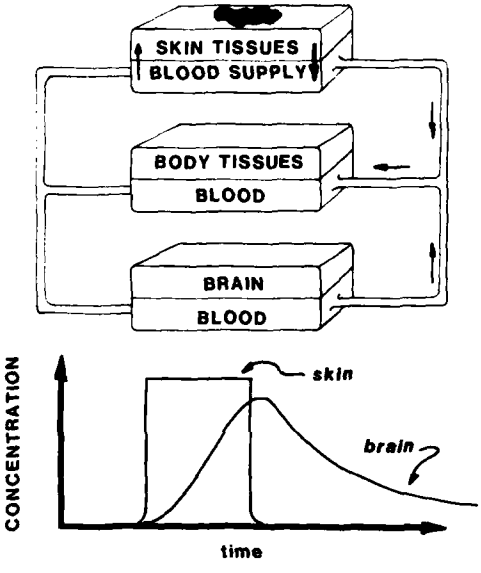

FIG. 11. Systemic toxicity resulting from dermal application. The heavy arrow from skin tissue to blood supply indicates the net movement to blood and then to remote tissues.

venous blood perfuses the lungs for oxygenation before reaching the arterial circulation. Without rigorously examining the mathematics, the concentration-time integral of the dose at organs remote from the application is expected to be linearly related to the applied dose so long as all processes are first order. The rapidity of absorption is dependent on a variety of factors, including permeability of the skin section, area of skin surface in contact with the chemical, and the integrity of the dermal barrier.

Irritation and direct dermal toxicity are troublesome, frequent occurrences in the industrial/occupational environment but are not usually fatal. Systemic effects following dermal application can be life-threatening depending on the tissue and the level of involvement. The potential for systemic toxicity after dermal exposure can be appreciated by referring to the list of threshold limit values (TLVs) for chemical substances (19). Many of these chemicals bear a skin notation indicating a substantial risk associated with skin exposure. The organophosphorus pesticides are important examples of chemicals with potentially lethal consequences following skin contact.

SYSTEMIC APPLICATION: DERMAL TOXICITY

Although systemic application with dermal toxicity is generally of less interest from the point of view of occupational and industrial toxicology, it gives the best opportunity to examine the influence of physiological factors on toxicokinetic behavior. The two primary routes of xenobiotic exposure, after skin contact, are inhalation and oral ingestion. Oral ingestion can also be an important route of intake for particulate material in inhaled air. Particulates are deposited on surfaces of the

upper airways, moved by mucociliary transport to the trachea, and swallowed. The various routes of administration are shown in Fig. 12.

In a U.S. Environmental Protection Agency (EPA) publication, there is a statement comparing inhalation and dermal exposure (5):

> In some respects, dermal toxicity studies are analogous to inhalation studies. In neither case is a fixed amount of test substance administered to the test species as it is in an injection or intubation study; but instead the animal is exposed to a concentration of the test substance. The actual dose received is a function of the amount of substance absorbed through the skin or lung tissue. For inhalation, the concentration of the chemical in the chamber is determined; for dermal toxicity the amount of material applied to a given surface area or the amount applied per unit of body weight is the expression of dose.

Actually, this point is equally valid for oral administration where the dose is expressed as the amount placed in the alimentary tract per kilogram of body weight. There is no guarantee of absorption, and concepts of applied dose and dose delivered to target tissues are separate and not necessarily related in any direct, linear fashion. It is apparent in Fig. 12 that multiple routes exist to limit bioavailability by these three routes. Some for the skin—evaporation, metabolism in skin, pulmonary elimination, etc.—have been mentioned. Inhalation is more like intraarterial injection with little opportunity for "presystemic clearance," and uptake varies depending on the solubility of the chemical in blood, the amount of metabolism of the xenobiotic *in vivo*, the rate and depth of respiration, preexisting lung disease, etc. For oral

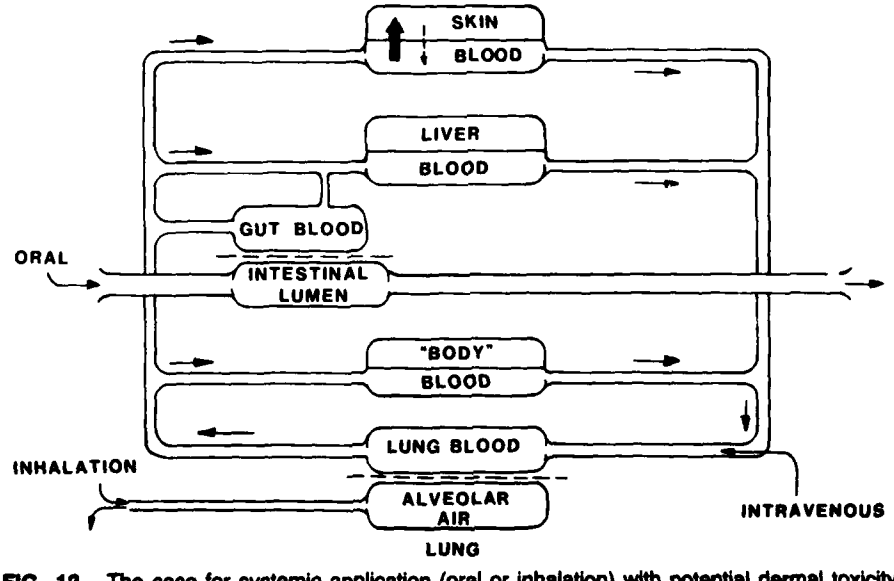

FIG. 12. The case for systemic application (oral or inhalation) with potential dermal toxicity from accumulation of the parent chemical or metabolite in skin tissue. Whether a chemical is placed on the skin, in the gut, or inspired in the air, there is no reason to assume *a priori* that all of the dose will be absorbed. Multiple physiological factors can impede delivery of the chemical from the site of application to a remote target tissue.

administration, absorption depends on feeding status, gut content, ability of the chemical to penetrate the membranes separating the gut lumen from the capillary blood supply, and other factors. "Presystemic" clearance can be by metabolism in gut flora and tissue, liver, and lung (4). Of these three routes of exposure, oral administration is unique in that the entire portal drainage perfuses the liver, which has large concentrations of xenobiotic-metabolizing enzymes. This gives rise to the opportunity for "first-pass effects," where very effective hepatic metabolism can essentially remove all chemical from perfusing blood at low concentrations. There is also opportunity for pulmonary first-pass effects as all venous blood must perfuse the pulmonary bed for oxygenation. The lung has a lower concentration of xenobiotic-metabolizing enzymes than liver but is perfused with the entire cardiac output (6). Lungs are also efficient in clearing certain biogenic amines by a process involving active transport mechanisms (22).

The interesting kinetic aspect of metabolism or active uptake is that these processes are capacity-limited (1). In the body they proceed at an accelerated rate because of the participation of small amounts of protein catalysts, both enzymes and carrier proteins. Because there is only a finite amount of enzyme or carrier, rates are complexly dependent on substrate concentration. *In vitro* the rate law for metabolism is given by

$$\text{Rate} = \frac{(\text{maximum rate})\ (\text{concentration})}{(\text{Michaelis constant}) + (\text{concentration})} \qquad [12]$$

At low values of concentration, the rate is first order.

$$\text{Rate} = \frac{(\text{maximum rate})\ (\text{concentration})}{(\text{Michaelis constant})} = k_m\ (C_s) \qquad [13]$$

At high concentration it is "pseudo-zero" order; that is, the rate appears to be independent of substrate concentration.

$$\text{Rate} = (\text{maximum rate}) = V_{max} \qquad [14]$$

This is the reason the processes are called capacity-limited. With a true first-order process, the rate increases without bounds as concentration increases. However, for the capacity-limited system, some maximum is reached which cannot be exceeded. The participation of capacity-limited processes has marked effects on pharmacokinetic behavior.

Other work in our laboratory has been on the metabolism of inhaled vapors. Although not directly related to skin toxicity, this research has relevance for understanding toxicokinetic principles as they relate to dermal effects. Consider the case where the metabolite is responsible for dermal toxicity and the parent chemical is delivered by inhalation. What relationship is expected between rate of metabolism and inhaled concentration, and what relationship is expected between concentration of parent chemical at target tissue and inhaled concentration?

We have evaluated the steady-state behavior of metabolized gases and vapors by several techniques. In one approach we simply expose rats for 6 hr and evaluate the blood concentrations at cessation of exposure (Fig. 13). For a chemical such as styrene (2,17), which has a blood:gas partition coefficient near 40, the relationship between blood and air styrene concentrations was not a simple linear dependence. In the region of first-order metabolism the proportionality constant is about 2.0, but after metabolism is saturated it increases to 10.0 or greater. Thus for the parent chemical both in the blood or at the target tissue and for the amount of metabolite formed, there is no simple relationship with inhaled concentration. This could lead to anomalous dose-response curves for any effect unless the effect is indexed to some proper measure of delivered dose.

These curves for styrene provide an example of nonlinear kinetic behavior. When all processes, including metabolism, are first order, the kinetics are said to be "linear," and the processes are directly and linearly porportional to concentration. When metabolism becomes saturated and of less than first order, the behavior is no longer linear and the relationship between inhaled concentration and target organ concentration becomes complex.

PHOTOTOXICITY

Phototoxicity, a toxicodynamic parameter, adds a new dimension to the evaluation of dermal effects. The intensity of this dermal effect is related to the concentration-time integral and the intensity of light. The development of toxicity usually follows an action spectrum related to the absorption characteristics of the compound, and the effect is regarded as the light-induced counterpart of primary irritation. In general, testing is conducted by direct dermal application followed by ultraviolet irradiation with a high-intensity light source. Phototoxicity could be expected following application to the skin or after systemic dosing and delivery to the skin.

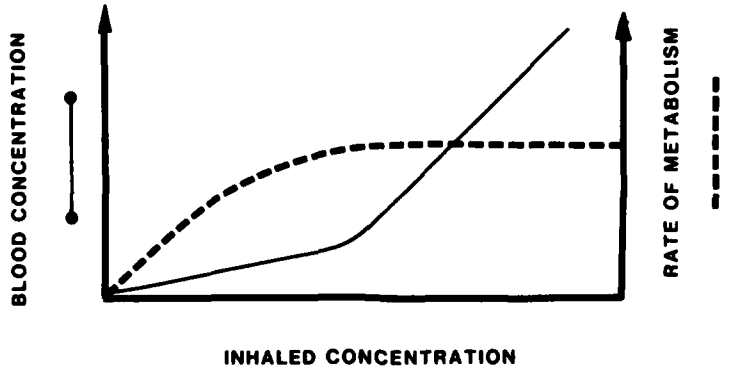

FIG. 13. Dependence of blood concentration and rate of metabolism of a soluble, well-metabolized, vapor-like styrene on inhaled concentration. The nonlinear behavior of both curves is a result of the capacity-limited nature of the enzymatic processes responsible for styrene metabolism.

For testing phototoxic potential, application to the skin is most likely to demonstrate the effect caused by high local concentrations. If a substance yields a metabolite that has phototoxic potential, it could be negative when applied to skin but positive if given systemically to increase the amount metabolized. Factors affecting phototoxicity at a particular skin site are also listed in the EPA monograph (5) and include:

> (1) both the quantity and location of the photosensitizer on the skin; (2) the capacity of the chemical to penetrate the skin through percutaneous absorption as well as trauma such as abrasion or sunburn; (3) the pH, the presence of enzymes and solubility conditions at the site of exposure; (4) the durations and intensity of exposure to activating radiation; (5) the depth of penetration of the activating radiation; (6) the humidity and ambient temperature; (7) the thickness of the horny layer of skin; and (8) the presence and degree of pigmentation.

IN VITRO METHODS FOR KINETICS

One area of emphasis in toxicokinetic research in our laboratory is on developing fairly complete physiological models of inhalation and percutaneous exposure. These routes are stressed because less is known about them compared to oral or intravenous routes, which are widely studied in drug pharmacokinetics research. In addition, they are the most important routes of exposure in the occupational environment.

The kinetic models used are intended to both explain observed kinetic data and be amenable to extrapolation. Extrapolation includes predicting behavior at one dose level from results at higher or lower dosages (i.e., predict conditions under which nonlinearities occur), predicting behavior for a route of administration other than those directly investigated, and predicting behavior in a species (usually man) based on results in laboratory test animals. Physiological models are better suited to these tasks than are conventional compartmental analyses (10). Finally, the kinetic models developed should be suited to predicting kinetic behavior if the proper combination of physiological and biochemical constants is known or can be readily determined. For percutaneous absorption the permeability constants can be determined *in vitro* using excised skin tissue and appropriate chambers (Fig. 14). The test material is placed on excised skin in a vehicle and its appearance in the bathing medium determined. A plot of concentration in the bath versus time has a lag, a period of increasing slope, and a final phase of constant slope. The plot of rate of uptake—the first derivative of this curve—gives the maximum uptake rate. The permeability constant, P (cm/hr), is the uptake rate (mg/cm^2/time) divided by applied concentration (3,9).

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Models of uptake from skin would require values for P, estimates of the area involved, and blood flows to the contact area (Fig. 9). A mass-balance differential equation is then written for the local skin area:

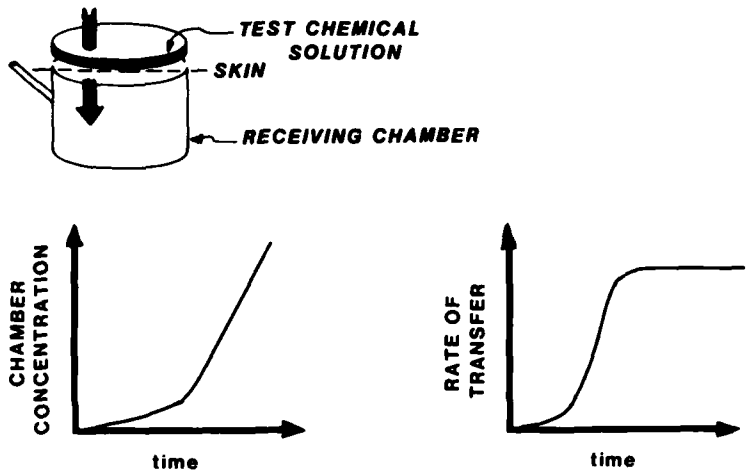

FIG. 14. *In vitro* methods to estimate permeability constants for skin tissues. The values for skin permeability obtained may be useful for physiological models to simulate dermal kinetics of substances and extrapolate such results to humans.

$$V_s \frac{d}{dt}(C_s) = Q_s \cdot C_{art} - Q_s(C_s/R_s) + P_A C_A - P_A C_S \qquad [15]$$

Where V_s is the volume of the local skin compartment, C_s is the concentration in skin, C_A is the concentration applied to the skin, and C_{art} and C_S/R_S are, respectively, arterial and venous blood concentrations in the skin. Q_S, R_S, and P_A are, respectively, blood flow to the local skin region, the partition coefficient for the chemical in skin tissue compared to blood, and the permeability constant for the chemical. This is the simplest form where C_A is assumed to remain constant. Other scenarios can be evaluated, e.g., ones which include evaporative loss or dermal metabolism. This description also assumes that there is little resistance to movement of chemical from skin tissues into blood. This behavior is called flow-limited, in contrast to diffusion-limited because blood flow is considered to be slow with respect to chemical diffusion across the membranes. For many chemicals, this may not be a valid assumption in skin tissues. Differential equations are then written for each explicit organ in the model and solved to simulate uptake from the skin and predict local tissue concentrations for various dermal exposure scenarios.

The target tissue doses associated with overt toxicity are usually easier to evaluate in studies of the oral, intraperitoneal, or inhalation toxicokinetics of a chemical. By comparison with the target tissue concentrations (or concentration-time integrals) that produce overt toxicity by other routes, the simulations of dermal uptake and distribution can be used to predict if skin absorption is likely to lead to hazardous tissue concentrations. Physiological models developed for test animals can be extrapolated to "estimate" human behavior based on the variation in physiological

and metabolic parameters from species to species. This extrapolative procedure is referred to as animal scale-up (7,8). Although this outlined approach to estimating dermal hazards of chemicals appears involved, it follows very closely the recommendation in the EPA dermatotoxicity volume (5) which suggests:

> Because of differences in permeability, and other variables such as the area of the patch and the amount and concentration of the test substance, it is extremely difficult to quantitatively predict the dermal effects of a chemical in man even after extensive animal studies. The same quality of information could be obtained by determining relative rates of percutaneous absorption of a substance and integrating this information with toxicokinetic studies by these other routes of administration.

Our approach is to integrate this information quantitatively through the vehicle of toxicokinetic simulation instead of qualitatively by indirect comparison. It is true, nonetheless, that considerable useful information is available simply by comparing the LD_{50} determined by oral, intravenous, and percutaneous routes of absorption.

SUMMARY

Toxicokinetic parameters determine the dose delivered to target tissues. Relationships between administered dose and concentration-time integrals at target tissues are often complexly related to dose, especially when one considers very large ranges of dose and participation of capacity-limited pathways. The skin is an interesting organ from the viewpoint of toxicokinetics because it is at once a target organ and an organ of delivery. This dichotomy of function leads to various combinations that illuminate those toxicokinetic concepts important for dermal toxicity and percutaneous absorption. Compartmental analysis of percutaneous hydrazine absorption shows the utility and meaning of lumped kinetic constants, whereas analysis of physiological models, even in a cursory way, gives anatomical meaning to the underlying physical reality of these constants. The very exercise of constructing a physiological model allows the experimentalist to conceptualize problems of incomplete absorption, presystemic clearance, and flow limitations for certain metabolic pathways. Extrapolation from kinetic data derived from other routes of administration using *in vitro* permeability constants to predict percutaneous hazard seems to be a promising application for toxicokinetic simulation.

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